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Total and *Toxocara canis* larval excretory/secretory antigen- and allergen-specific IgE in atopic and non-atopic dogs

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Inhaltsverzeichnis

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Total and *Toxocara canis* larval excretory/secretory antigen- and allergen-specific IgE in atopic and non-atopic dogs

Summary:

Background – Total IgE concentrations are higher in dogs than in humans. Persistent *Toxocara canis* larval infections are prevalent in dogs and are associated with substantial specific antibody reactions. A correlation, however, between total IgE and *T. canis*-specific antibody levels has not yet been evaluated in dogs.
Objectives – To determine the relationship between total IgE, *T. canis*-specific IgG and IgE, as well as allergen-specific IgE levels in atopic and non-atopic dogs and to evaluate possible confounding factors.
Animals – Sera of 30 atopic and 30 non-atopic client-owned dogs.
Methods – Antibody levels were evaluated by ELISA.
Results – Total IgE, *T. canis*-specific antibody and allergen-specific IgE levels were significantly higher in non-atopic compared to atopic dogs. A positive correlation was demonstrated between *T. canis*-specific IgG and *T. canis*-specific IgE, *T. canis*-specific IgG and total IgE, *T. canis*-specific IgE and total IgE, as well as allergen-specific IgE and total IgE.
Conclusions – *T. canis*-specific IgE appears to be a major component of total IgE in dogs. It is speculated that *T. canis* infection may have a protective effect on the development of canine atopic dermatitis and/or that elevations in serum total IgE level are often not associated with atopic dermatitis.

Canine Atopic Dermatitis – Total IgE – *T. canis*-specific IgG – *T. canis*-specific IgE – Allergen-specific IgE

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Zusammenfassung

Hintergrund – Die Gesamt-IgE-Konzentrationen sind bei Hunden höher als bei Menschen. Persistierende *Toxocara canis* Larveninfektionen sind bei Hunden weit verbreitet und mit starken spezifischen Antikörperreaktionen assoziiert. Eine Korrelation zwischen Gesamt-IgE- und *T. canis*-spezifischen Antikörper-Werten wurde bei Hunden jedoch bisher nicht evaluiert.
Ziele – Die Beziehung zwischen Gesamt-IgE-, *T. canis*-spezifischen IgG- und IgE-, sowie Allergen-spezifischen IgE-Werten bei atopischen und nicht-atopischen Hunden zu ermitteln und mögliche Einflussfaktoren zu evaluieren.
Tiere – Sera von 30 atopischen und 30 nicht-atopischen Hunden in Privatbesitz.
Methoden – Die Antikörper-Werte wurden mittels ELISA evaluiert.
Ergebnisse – Die Gesamt-IgE-, *T. canis*-spezifischen Antikörper- und Allergen-spezifischen IgE-Werte waren signifikant höher bei nicht-atopischen im Vergleich zu atopischen Hunden. Eine positive Korrelation wurde festgestellt zwischen *T. canis*-spezifischem IgG und *T. canis*-spezifischem IgE, *T. canis*-spezifischem IgG und Gesamt-IgE, *T. canis*-spezifischem IgE und Gesamt-IgE, sowie Allergen-spezifischem IgE und Gesamt-IgE.
Schlussfolgerungen – *T. canis*-spezifisches IgE scheint bei Hunden eine Hauptkomponente des Gesamt-IgEs zu sein. Es wird spekuliert, dass eine *T. canis*-Infektion eine schützende Wirkung auf die Entwicklung der atopischen Dermatitis bei Hunden haben kann und/oder dass Erhöhungen des Gesamt-Serum-IgE-Wertes oft nicht mit atopischer Dermatitis assoziiert sind.

Canine Atopische Dermatitis – Gesamt-IgE – *T. canis*-spezifisches IgG – *T. canis*-spezifisches IgE – Allergen-spezifisches IgE

Veterinary Dermatology

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Total and *Toxocara canis* larval excretory/secretory antigen- and allergen-specific IgE in atopic and non-atopic dogs

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Background – Total IgE concentrations are higher in dogs than in humans. Persistent *Toxocara canis* larval infection is prevalent in dogs and is associated with substantial specific antibody reactions. A correlation, however, between total IgE and *T. canis*-specific antibody levels in dogs has not been evaluated.

Objectives – To determine the relationship between total IgE, *T. canis*-specific IgG and IgE, and allergen-specific IgE levels in atopic and non-atopic dogs, and to evaluate possible confounding factors.

Animals – Sera of 30 atopic and 30 non-atopic client-owned dogs.

Methods – Total IgE, *T. canis*-specific antibody and allergen-specific IgE levels were evaluated by ELISA.

Results – Total IgE, *T. canis*-specific antibody and allergen-specific IgE levels were significantly higher in non-atopic compared to atopic dogs. A positive correlation was demonstrated between *T. canis*-specific IgG and *T. canis*-specific IgE; *T. canis*-specific IgG and total IgE; *T. canis*-specific IgE and total IgE; and allergen-specific IgE and total IgE. No differences were detected on the basis of age, gender, vaccination status; deworming or season between atopic and non-atopic dogs. Previous immunomodulatory treatment and cause of atopy did not influence antibody levels of atopic dogs.

Conclusions – *Toxocara canis*-specific IgE appears to be a major component of total IgE in dogs. Total and *T. canis*-specific IgE levels are higher in non-atopic compared to atopic dogs. It is speculated that *T. canis* infection may have a protective effect against the development of canine atopic dermatitis and/or that elevations in total serum IgE level are often not associated with atopic dermatitis.

Introduction

Immunoglobulin E (IgE) is associated with atopic disease and parasitic infection.[1] IgE antibodies can, however, be expressed in the absence of antigen stimulation. Total IgE is composed of IgE from three different origins: IgE produced independent of T-cell signalling; parasite-specific IgE produced subsequent to host–parasite interaction; and allergen-specific IgE produced subsequent to specific sensitization in predisposed individuals.[2] Canine atopic dermatitis (cAD) is a common, chronic and pruritic inflammatory skin disease of dogs.[3] The diagnosis is based on history, clinical signs and the elimination of other causes of pruritus.[4] Although cAD can be associated with high levels of allergen-specific IgE, serological allergen tests are not recommended for the diagnosis because healthy dogs can have elevated allergen-specific IgE levels.[5–7]

In humans, increased total serum IgE level can be a useful marker for the confirmation of atopy especially in children from industrialized countries. Elevations of total IgE levels greater than 0.24 μg/mL or 0.54 μg/mL are considered consistent with the diagnosis of atopic disease.[8,9] Non-atopic humans infected with endoparasites, however, also can exhibit very high total IgE levels. For example, humans infected with *Toxocara canis* can exhibit total serum IgE levels >1.2 μg/mL.[10,11]

Total IgE concentrations in healthy dogs are much higher compared to healthy humans.[12] The maximum total IgE level in healthy dogs ranges from 0.5 to 505 μg/mL (Table 1). Higher levels of total IgE in dogs compared to humans could be associated with more frequent helminth infections of dogs.[13,14] *Toxocara canis* is a common intestinal nematode of dogs;[15–17] it can cause high total IgE levels in infected humans.[10,11] The prevalence of patent *T. canis* infection in dogs depends on various

Abbreviations: ASIT, allergen-specific immunotherapy; cAD, canine atopic dermatitis; ELISA, enzyme linked immunosorbent assay; FIAD, food-induced atopic dermatitis; HERBU, Heska Epsilon Receptor Binding Units; OD, optical density; RIA, radioimmunoassay; RID, radial immunodiffusion; WHWT, West Highland white terrier; WSS, Welsh springer spaniel.

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Table 1. Total serum IgE assays in healthy and atopic dogs – comparison of published studies

Total IgE healthy dogs	Total IgE atopic dogs	Method	Reference
25–410 μg/mL*	85–550 μg/mL*	RIA	25
47,000 μg/mL†	31,000 μg/mL§	RID	26
<0.0005–2 μg/mL*		ELISA	27
0.5 μg/mL†	0.3 μg/mL§	ELISA	28
0–12 μg/mL*		ELISA	29
0.2–5.7 μg/mL*	1.4–4.4 μg/mL*	ELISA	30
0.05–0.5 μg/mL*		ELISA	31
0.4 μg/mL†	0.3 μg/mL§	ELISA	32
0.7–6 μg/mL*	7–34 μg/mL*	ELISA	33
1–35 μg/mL*	4–20 μg/mL*	ELISA	14
0–138 μg/mL*		ELISA	34‡
0.5–505 μg/mL*	0.4–743 μg/mL*	ELISA	35

RIA radioimmunoassay, RID radial immunodiffusion, ELISA enzyme linked immunosorbent assay.
*Range. †Mean. ‡Study in wolves. §mean3 in allergic dogs.

factors (age, origin, locality of the dog) and varies from 3 to 34% in Europe.[18–23] Furthermore, the prevalence of dogs with *T. canis*-specific antibodies to larval excretory/secretory (E/S) antigen is much higher. This arises from infection with persistent *T. canis* larvae in the absence of patent infection at the time of evaluation.[24]

It is well established that total IgE concentration does not correlate with the development of atopic dermatitis in dogs. There is either no difference between total IgE values between atopic and healthy dogs or levels can be higher in healthy dogs (Table 1). It has been proposed that helminth infection and/or helminth-specific IgG or IgE antibodies may play a protective role against the development of cAD. This could potentially explain why, in some studies, healthy dogs have a higher total serum IgE concentration compared to atopic dogs.

In the present study, the interaction between total IgE, *T. canis*-specific IgG and *T. canis*-specific IgE levels in the sera of atopic and non-atopic dogs was evaluated. In addition, the influence of these antibody levels on allergen-specific IgE values and various confounding factors affecting total IgE and *T. canis*-specific IgG and IgE levels were evaluated.

Materials and methods

Animals

The study was performed using sera collected consecutively from 60 (30 atopic and 30 non-atopic) dogs presenting to the dermatology unit at the Vetsuisse Faculty, University of Zurich. The diagnosis of atopic dermatitis was based on each dog fulfilling at least five criteria, after ectoparasite infections and bacterial or yeast dermatitis were excluded.[36] To confirm or exclude food-induced atopic dermatitis (FIAD), an elimination diet was conducted and dogs that demonstrated an improvement during the trial were challenged with the previously fed foods. If the clinical signs improved during the dietary trial and relapsed upon food challenge, then FIAD was confirmed. If the dog did not respond to the elimination diet or demonstrated only a partial improvement of the clinical signs, an intradermal test with Greer allergens (Greer Veterinary Laboratories; Lenoir, NC, USA) and/or a serological allergen test (Allercept™ system, Heska AG; Fribourg, Switzerland) was performed.

For each serum sample collected from an atopic dog, a control serum sample from a healthy donor of similar age and breed (using the breed group and section as designated by the Fédération Cynologique Internationale; http://www.fci.be/en/) was collected (Table 2). The control dogs had no history of allergic skin disease or any other disease affecting the immune system and were not receiving any immunomodulatory drugs (ciclosporin, glucocorticoids or oclacitinib).

Factors influencing total IgE and *Toxocara canis* L3 E/S-specific antibodies

Age, sex, frequency of deworming, vaccination status, estrous cycle phase, season of sample collection, concurrent immunomodulatory treatment and cause of the atopic disease (i.e. food or environmental allergens) was recorded for each dog. Samples collected during the months with low pollen loads (October to March) were compared to those collected during months with higher pollen loads (April to September) to determine the effect of seasonal influence. Dogs dewormed at least four times a year and less than three months prior to serum collection were compared to the dogs not fulfilling these criteria. Within the group of atopic animals, the dogs treated with systemic immunomodulatory drugs (ciclosporin, glucocorticoids, oclacitinib) were compared to dogs receiving no treatment or only topical medication. Additionally, the dogs treated with allergen-specific immunotherapy (ASIT) were compared to the dogs that did not receive ASIT.

Determination of total IgE

Total serum IgE concentrations were evaluated using a commercial dog IgE ELISA quantitation kit (Bethyl Laboratories; Montgomery, AL, USA). To validate these data, ten additional dog sera analysed in a previous study were measured using this ELISA methodology and the results compared.[35] The measured total IgE concentrations were comparable with a correlation of $r = 0.95$ and a paired *t*-test *P*-value of 0.75 (data not shown).

Determination of *Toxocara canis* L3 E/S-specific IgG

IgG antibodies against larval *T. canis* E/S antigen were evaluated using a method described previously.[24] *Toxocara canis* L3 E/S antigen was diluted 1:500 in 0.1 M carbonate-bicarbonate (Na_2CO_3-$NaHCO_3$) buffer (pH 9.6) containing 0.02% sodium azide (NaN_3) and used to coat 96-well microtitre plates (Nunc; Roskilde, Denmark) overnight at 4°C. The plates were washed (0.9% NaCl, 0.3% Tween 20) before being blocked for 30 min at 37°C with PBS-HT [phosphate-buffered saline (pH 7.2) containing 0.05% bovine haemoglobin (Fluka; Buchs, Switzerland), 0.3% Tween 20 and 0.02% NaN_3]. The dog sera were diluted 1:200 in PBS-HT and incubated at 37°C for 1 h. After washing the plates as mentioned above, alkaline phosphatase labelled goat anti-dog IgG(γ) antibody (Kirkegaard & Perry Laboratories; Gaithersburg, MD, USA) at a dilution of 1:750 in PBS-HT was added to the plate and incubated for 1 h at 37°C. Following a final wash, the substrate (1 mg/mL *p*-nitrophenyl phosphate (Sigma-Aldrich; St. Louis, MO, USA) in 0.05 M Na_2CO_3-$NaHCO_3$buffer (pH 9.8) plus 1 mM MgCl) was added. After 10 min, the optical density (OD) was measured at 405 nm using a Multiscan RC ELISA reader (Thermo Labsystems; Helsinki, Finland).

On each plate, we included five negative control sera of specific pathogen free (SPF) dogs and two positive control sera of dogs with proven *T. canis* infection, to allow the interassay calibration. The cut-off for *T. canis* larval E/S-specific IgG at OD <0.026 was determined by a receiver operating characteristic (ROC) curve.

Determination of *Toxocara canis* L3 E/S-specific IgE

IgE-specific antibodies against larval *T. canis* E/S antigen were evaluated using a similar methodology: plates were coated, blocked and sampled as described above for *T. canis* L3 E/S-specific IgG analysis. For detection of IgE levels, a biotinylated anti-canine IgE monoclonal antibody 5.91 (provided by Bruce Hammerberg, North Carolina State University, Raleigh, NC, USA) in a 0.25 μg/mL dilution with PBS containing 0.05% Tween 20 (PBS-T) was added for 2 h at 37°C. Following washing, alkaline phosphatase-conjugated streptavidin (Thermo Scientific; Waltham, MA, USA) was added at a dilution of 1:5,000 in PBS-T and incubated for 1 h at 37°C. After a final wash, the plates were developed as described above and after 5 min, the OD values were read at 405 nm.

Table 2. Clinical characteristics of healthy and atopic dogs sampled for serological studies of Toxocara

Atopic dogs					Healthy dogs			
No.	Breed	Gender	Age*	Diagnosis	No.	Breed	Gender	Age*
1	Toy poodle	mn	4	cAD	31	Poodle	fn	1
2	Yorkshire terrier	fn	14	Undetermined	32	Yorkshire terrier	m	17
3	Rhodesian ridgeback	m	3	FIAD	33	Rhodesian ridgeback	f	6
4	English bulldog	f	6	cAD	34	Boxer	fn	5
5	Bull terrier	m	4	cAD + FIAD	35	English bulldog	fn	3
6	Pomeranian	fn	12	FIAD	36	Pomeranian	fn	11
7	Airedale terrier	f	10	cAD	37	Parson Jack Russell terrier	fn	13
8	Golden retriever	m	11	cAD + FIAD	38	Golden retriever	m	10
9	Picard	f	4	cAD	39	German shepherd dog	f	4
10	Jack Russell terrier	mn	9	cAD	40	Jack Russell terrier	f	10
11	German shepherd dog	fn	4	cAD	41	German shepherd dog	mn	5
12	Labrador retriever	m	10	cAD	42	Labrador retriever	mn	7
13	English bulldog	m	1	FIAD	43	Boxer	f	2
14	Labrador cross	fn	7	cAD	44	Labrador retriever		5
15	French bulldog	f	1	cAD	45	French bulldog	m	2
16	Labrador retriever	fn	5	cAD	46	Labrador retriever	fn	4
17	Newfoundland	m	4	cAD	47	Leonberger	f	5
18	German shepherd dog	fn	6	cAD	48	German shepherd dog	f	6
19	Spanish mastiff	fn	8	cAD + FIAD	49	Newfoundland	fn	7
20	Beagle	mn	10	cAD	50	Beagle	fn	10
21	Bernese cattle dog	f	0	FIAD	51	Bernese cattle dog	m	0
22	Argentine mastiff	f	1	cAD	52	Boxer	m	1
23	Pitbull mix	m	2	cAD + FIAD	53	Rottweiler	fn	3
24	WHWT	fn	11	Undetermined	54	WHWT	fn	7
25	Cross-breed	fn	4	FIAD	55	Cross-breed	mn	4
26	Cocker spaniel	fn	10	cAD + FIAD	56	Cocker spaniel	f	7
27	Cocker spaniel	m	11	cAD	57	WSS	fn	10
28	Labrador retriever	mn	3	cAD	58	Labrador retriever	f	3
29	Pug	m	1	cAD	59	Pug	fn	3
30	Basset hound	mn	9	cAD	60	Beagle	mn	8

cAD canine atopic dermatitis, FIAD food-induced atopic dermatitis, f female, m male, n neutered, WHWT West Highland white terrier, WSS Welsh springer spaniel.
*Rounded.

Determination of allergen-specific IgE

Allergen-specific IgE levels in sera samples from 20 atopic and 13 non-atopic dogs were measured by Heska AG in Fribourg, Switzerland. The remaining sera of 10 atopic and 17 non-atopic dogs were not analysed due to insufficient serum quantities. The allergen-specific IgE concentrations for 48 different antigens were measured with the Heska Allercept™ system and expressed in Heska Epsilon Receptor Binding Units (HERBUs).[37] For each dog the HERBUs of the 48 allergens were added to calculate the individual sum of allergen-specific IgE. These allergen-specific IgE sums were then compared to the total IgE concentrations in both groups. In addition, the ratio of allergen-specific IgE sum to total IgE concentration was calculated for each dog. These ratios, as well as the *Dermatophagoides farina* (Df)- and *Dermatophagoides pteronyssinus* (Dp)-specific IgE values, were compared between the atopic and non-atopic group.

Statistical methods

Statistical analyses were conducted using SPSS v22.0 (IBM Corp; Armonk, NY, USA). The Kolmogorov test demonstrated that the total IgE, *T. canis*-specific IgG and IgE, and allergen-specific IgE values were not normally distributed. A nonparametric test (Spearman's rank correlation coefficient) was therefore used to evaluate the correlations between the antibody levels. ANOVA was used to compare the antibody levels between the atopic and non-atopic dogs, and to determine the correlations between the antibody levels and various confounding factors, such as age, gender, deworming, season and vaccination status. Following ANOVA, a *post hoc* Student's *t*-test was conducted. When a statistically significant difference was detected, a Wilcoxon–Mann–Whitney U-test was additionally performed for confirmation. The level of significance was set at $P < 0.05$.

Results

Study population

Thirty atopic (male:female ratio 14:16; mean age 6.2 years) and 30 non-atopic (male:female ratio 9:20; mean age 6 years) dogs were included (Table 2). Of 30 atopic dogs, 18 were diagnosed with cAD and 10 with FIAD. In five dogs with FIAD, the clinical signs did not resolve completely during dietary restriction. Furthermore, in two dogs the incriminated allergens were not identified because the elimination diet was not completed and environmental allergen testing was negative.

Total IgE

The total IgE concentrations were overall very high and significantly (Student's *t*-test, $P = 0.02$; Wilcoxon–Mann–Whitney U-test, $P = 0.002$) increased in the non-atopic (range 0.4–412.1 μg/mL, mean 64.5 μg/mL, median 47.3 μg/mL; Table 3) compared to the atopic dogs (range 0.5–120.8 μg/mL, mean 22.6 μg/mL, median 12.3 μg/mL; Table 3). There was a wide range of total IgE in the atopic and non-atopic dogs, with overlap in the levels between the groups. (Table 3).

Toxocara canis L3 E/S-specific IgG

Toxocara canis-specific IgG values above the cut-off were detected in 83.3% (25 of 30) of the atopic and 96.7% (29 of 30) of the non-atopic dogs. In non-atopic individuals the

Table 3. Antibody levels of *Toxocara canis*-specific IgG and IgE, total IgE and allergen-specific IgE in atopic and healthy dogs

Atopic dogs						Healthy dogs					
No.	*T. canis* IgG (OD)	*T. canis* IgE (OD)	Total IgE (μg/mL)	Sum of allergen IgE (HERBU)	HERBU/ Total IgE	No.	*T. canis* IgG (OD)	*T. canis* IgE (OD)	Total IgE (μg/mL)	Sum of allergen IgE (HERBU)	HERBU/ Total IgE
1	0.013	0.014	42.4			31	0.05	0.014	0.7		
2	0.245	0.989	120.8			32	0.845	0.542	55.6		
3	0.021	0.011	1	1	0.001	33	0.297	0.127	54.7		
4	0.037	0.012	10.7	47	0.004	34	0.785	0.055	69.6		
5	0.017	0.012	1.1	54	0.049	35	0.093	0.022	43.4		
6	0.057	0.012	0.8	67	0.088	36	0.165	0.03	0.6	29	0.049
7	0.178	0.012	0.5			37	0.578	0.311	76.8	1,191	0.015
8	0.11	0.147	9.8	4,557	0.465	38	0.053	0.024	66.7		
9	0.198	0.049	13.3			39	0.44	0.101	44.6	402	0.009
10	0.263	0.073	18			40	0.894	0.395	46.4		
11	0.093	0.031	52.6			41	0.392	0.023	6.1		
12	0.163	0.018	5.7	834	0.145	42	0.588	0.078	34.8		
13	0.137	0.035	49.1	231	0.005	43	0.478	0.692	52		
14	0.024	0.012	0.8	79	0.102	44	0.652	0.66	78.4		
15	0.45	0.114	8	1,578	0.197	45	0.041	0.035	17.2		
16	0.127	0.063	42.7	605	0.014	46	0.365	0.043	7.8	1,459	0.186
17	0.48	0.044	51.8	593	0.011	47	0.143	0.051	12.1	5,896	0.488
18	0.122	0.019	5.6			48	0.324	0.362	48.3	2,605	0.054
19	0.269	0.128	11.8			49	1.161	0.362	62.9	4,029	0.064
20	0.655	0.408	26.8	1,002	0.037	50	0.605	0.304	69.8	2,791	0.04
21	0.098	0.015	7.2	598	0.083	51	0.02	0.013	0.4	356	0.861
22	0.883	0.012	0.6	1,030	1.861	52	0.042	0.019	23.9	2,044	0.086
23	0.674	0.685	84.4	4,006	0.047	53	1.222	2.188	327.3		
24	0.111	0.046	16.6			54	0.323	0.192	56.1		
25	0.174	0.016	12.3	17	0.001	55	1.037	0.467	92.7	2,314	0.025
26	0.07	0.063	28.1	2,542	0.091	56	0.547	0.55	95.8		
27	0.037	0.012	12.9	249	0.019	57	0.954	0.149	33.8		
28	0.331	0.071	5.1	1,048	0.204	58	0.625	1.519	412.1	2,512	0.006
29	0.023	0.011	12.3			59	0.091	0.016	13.2		
30	0.157	0.037	26.1	2,303	0.088	60	0.165	0.154	32	2,492	0.078

OD optical density, HERBU Heska Epsilon Receptor Binding Units.

OD values ranged from 0.02 to 1.22 (mean 0.47, median 0.42; Table 3) and in atopic dogs from 0.01 to 0.88 (mean 0.21, median 0.13; Table 3). This difference was statistically significant (Student's *t*-test, $P = 0.004$; Wilcoxon–Mann–Whitney U-test, $P = 0.004$).

Toxocara canis L3 E/S-specific IgE

Toxocara canis-specific IgE values were significantly higher (Student's *t*-test, $P = 0.04$; Wilcoxon–Mann–Whitney U-test, $P = 0.002$) in non-atopic (range 0.01–2.19, mean 0.32, median 0.14; Table 3) than in atopic dogs (range 0.01–0.99, mean 0.11, median 0.03; Table 3).

Correlations between total IgE and *Toxocara canis* L3 E/S-specific antibodies

There was a significant ($P = 0.01$) positive correlation between *T. canis*-specific IgG and *T. canis*-specific IgE (76%), *T. canis*-specific IgG and total IgE (53%), as well as *T. canis*-specific IgE and total IgE (76%) in atopic and non-atopic dogs.

Factors influencing total IgE and *Toxocara canis* L3 E/S-specific antibodies

There were no statistically significant differences in total IgE concentrations or *T. canis*-specific IgG and IgE levels within the atopic and non-atopic groups with respect to age, gender, frequency of deworming, vaccination status and season of sample collection. Additionally, there was no association with the presence of FIAD, immunomodulatory drug therapy or recent desensitization within the atopic group. All the dogs with very high total IgE values had been dewormed at least four times a year and less than three months before blood collection. Five dogs from the non-atopic group (dogs 33, 39, 43, 48 and 58) were in estrus at the time of blood collection, but only one (Dog 58) had a high total IgE concentration (see Table S1).

Allergen-specific IgE

The total allergen-specific IgE levels were significantly ($P = 0.027$) higher in the non-atopic (range 29–5,896 HERBU, mean 2,163 HERBU, median 2,314 HERBU; Table 3) than the atopic dogs (range 1–4,557 HERBU, mean 1,072 HERBU, median 602 HERBU; Table 3). Furthermore, a positive correlation between total IgE concentration and the sum of all allergen-specific IgE ($r = 0.56$, $P = 0.002$) was evident in both groups. Conversely, there was no statistically significant difference in the ratio of total allergen-specific IgE to total IgE concentration or the Df- and Dp-specific IgE concentrations between atopic and non-atopic dogs.

Discussion

This study demonstrates that levels of total IgE, *T. canis*-specific IgG, *T. canis*-specific IgE and allergen-specific

IgE are significantly increased in healthy dogs compared to dogs with cAD. Furthermore, *T. canis*-specific IgE levels correlated with total IgE concentrations, suggesting that *T. canis*-specific IgE is a major component of total IgE in dogs.

Serum total IgE levels have been evaluated in healthy dogs. Canine total IgE concentrations reportedly range from undetectable to 505 μg/mL (Table 1) with values progressively increasing over time. In 1997, IgE levels in the range of <0.0005–2 μg/mL were reported, whereas 15 years later, the range was higher at 0.5–505 μg/mL.[27,35]

Dogs usually have higher total serum IgE concentrations than humans.[12] This could be explained by the natural behaviour of dogs during daily outdoor activities, and the more frequent exposure to and infection with parasites, particularly nematodes.[13,14] This study demonstrated that 90% of dogs, including those undergoing regular deworming, had a positive *T. canis*-specific IgG value. This suggests that dogs in Switzerland have sufficient exposure to *T. canis* to develop an antibody-mediated immune response. Given that the majority of dogs with a positive *T. canis*-specific IgG value also had an increased *T. canis*-specific IgE value, and that *T. canis*-specific IgE and total IgE levels positively correlated, our results could suggest that *T. canis*-specific IgE is a major component of total serum IgE in dogs. In humans, infection with nematodes, including *Toxocara* spp., can markedly increase total IgE levels.[10,11,38–40] This study also suggests that the absence of regular deworming does not explain the high total IgE levels detected in dogs. However, this could be explained by the observation that under experimental conditions, dogs repeatedly inoculated with embryonated *T. canis* eggs and treated with milbemycin still demonstrate a strong antibody response against *T. canis* L3 E/S antigen without developing patent infections.[24] Deworming does not eliminate extra-intestinal larval infection and dogs can be re-infected soon after treatment.[17,22,41] Furthermore, helminth-specific IgE and IgG levels may persist at an elevated level for a significant period of time even after anthelmintic therapy, as demonstrated in humans.[39,42]

In the majority of previously published studies total IgE concentrations between atopic and healthy animals did not differ (Table 1). In humans, by contrast, high total IgE levels are evidence for atopic disease in patients living in industrialized countries.[8,9,43] Non-atopic humans living in helminth-endemic areas, however, often have total IgE levels elevated above those of healthy people in developed western countries.[40,44] It is speculated that in dogs the lack of correlation between total IgE levels and atopy could be explained by the frequent infection with helminths resulting in higher total IgE levels masking more subtle increases in allergen-specific IgE.[7,45] This is contradicted by other studies demonstrating that atopic dogs have significantly higher total IgE levels than healthy dogs.[33]

The present study demonstrated significantly higher total IgE concentrations in non-atopic compared to atopic dogs; this has been demonstrated previously only in two other studies.[35,46] Increased IgE levels in healthy dogs could be the result of two things: the presence of anti-IgE antibodies in atopic dogs, which could interfere with the detection of IgE due to the formation of antibody complexes;[27,47] or the presence of helminth infections and/or helminth-specific antibodies protecting dogs against the development of atopy. The latter is supported by the present study in the positive correlation between *T. canis*-specific IgE and total IgE levels. The protective effect of *T. canis* could arise subsequent to *T. canis*-specific IgE binding to the surface and enhancing survival time of mast cells.[48] In the absence of exposure to Toxocara, mast cells may bind allergen-specific IgE in place of parasite related IgE; this may make some dogs more prone to showing signs of allergic skin disease. The protective effect of *T. canis* also could be explained by an immunosuppressive mechanism of helminth proteins.[49]

In the present study, non-atopic dogs also demonstrated higher values of *T. canis*-specific IgG than atopic dogs. It is feasible that healthy dogs may have been exposed more frequently to *T. canis* than atopic dogs, although given the selection criteria for this study, this seems less likely. A more plausible explanation is that healthy dogs produce more antibodies after exposure to *T. canis* compared to atopic dogs and that high levels of *T. canis*-specific serum immunoglobulins protect against the development of cAD. In recent years, the 'hygiene' hypothesis has suggested that the increase in allergic diseases in humans from industrialized countries can be explained by reduced exposure to infectious agents, symbiotic micro-organisms and parasites during the first years of life.[50] In this context, helminth parasitism has been described to have both positive and negative effects on the development of atopic diseases in humans.[51–53] It has been speculated that the response to parasite exposure varies depending on the species involved, the chronicity of the parasite infection, the parasite burden and/or the age of the infected individual.[54,55]

A significant positive correlation between the ratio of allergen-specific IgE to total IgE and the outcome of food challenge has been reported in humans with suspected milk, egg and wheat allergy.[56] In humans allergic to cat and house dust mite antigens, the ratio negatively correlated with total IgE concentrations, even though there was a tendency to higher allergen-specific IgE levels with increasing total IgE concentrations among the patients with a positive IgE test.[57] No significant difference was detected in the ratio of allergen-specific IgE to total IgE between atopic and non-atopic dogs in the present study. Besides significantly higher total IgE and allergen-specific IgE levels, non-atopic dogs also showed increased *T. canis*-specific IgE values. This raises the question as to whether some helminth-specific IgE may recognize allergens and lead to an overestimation of allergen-specific IgE levels in allergen tests. In humans living in helminth-endemic areas, allergen-specific IgE serologic testing has limited diagnostic value for allergic disease.[58] However, allergen-specific IgE values were measured in only 20 atopic and 13 non-atopic dogs in this study, and therefore the results should be interpreted cautiously.

There are intrinsic and extrinsic factors that reportedly influence total or allergen-specific IgE concentrations in the sera of dogs,[14,31,59–62] although not all studies

support these findings.[29,35,46] The present study did not demonstrate any correlation between age, gender, vaccination status or recent desensitization and total IgE concentrations. No correlation between the season or previous treatment with immunomodulatory drugs and total IgE levels in dogs was demonstrated.

In summary, the present study compared total IgE, *T. canis*-specific IgG, *T. canis*-specific IgE and allergen-specific IgE levels, in atopic and non-atopic dogs, and demonstrated that total IgE concentrations, *T. canis*-specific IgG and IgE levels, and total allergen-specific IgE values are increased in non-atopic dogs. Furthermore, this study demonstrated a positive correlation between *T. canis*-specific IgG and *T. canis*-specific IgE; *T. canis*-specific IgG and total IgE; *T. canis*-specific IgE and total IgE; and total IgE and allergen-specific IgE. This could suggest that *T. canis*-specific IgE is a major component of total IgE in dogs. Furthermore, high quantities of parasite-specific IgE could mask subtle but significant differences in total IgE concentration between allergic and healthy dogs. It is hypothesized that nematode infection could have a protective role against the development of atopic dermatitis in dogs.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1. Factors influencing total IgE and *Toxocara canis* L3 E/S-specific antibodies in atopic and healthy dogs.

Résumé

Contexte – Les concentrations totales d'IgE sont plus élevées chez le chien que chez l'homme. L'infection larvaire à *Toxacara canis* est prévalente chez le chien et est associée à une forte réaction d'anticorps spécifiques. Une corrélation entre les IgE totaux et les taux d'anticorps spécifiques de *T. canis* n'a cependant pas été évaluée chez le chien.

Objectifs – Déterminer la relation entre les IgE totaux, les IgE et IgG spécifiques de *T. canis* et les taux d'IgE spécifiques d'allergènes chez le chien atopique et non atopique et d'évaluer les facteurs possibles d'-terférence.

Sujets – Les sera de 30 chiens atopiques et 30 chiens non-atopiques de propriétaires.

Méthodes – Les taux totaux d'IgE, d'anticorps spécifiques de *T. canis* et d'IgE spécifiques d'allergènes ont été évalués par ELISA.

Résultats – Les IGE totaux, les anticorps spécifiques de *T. canis* et les taux d'IgE spécifiques d'allergène étaient significativement plus élevés chez les chiens non atopiques comparés aux atopiques. Une

corrélation positive a été démontrée entre les IgG spécifiques de *T. canis* et les IgE spécifique de *T. canis*, les IgG spécifiques de *T.canis* et les IgE totaux, les IgE spécifiques de *T.canis* et les IgE totaux et les IgE spécifiques d'allergènes et les IgE totaux. Aucune différence n'a été détectée sur la base de l'âge, du genre, de la vaccination, de la vermifugation ou de la saison entre les chiens atopiques et non-atopiques. Des traitements immunomodulateurs antérieurs et les causes de l'atopie n'avaient pas d'influence sur les taux d'anticorps des chiens atopiques.
Conclusions – Les IgE spécifiques de *Toxocara canis* semblent être un composant majeur des IgE totaux chez le chien. Les taux d'IgE spécifiques de *T. canis* et totaux sont plus élevés chez les chiens non-atopiques comparés aux chiens atopiques. Il est supposé que l'infection par *T.canis* pourrait avoir un effet contre le développement de la dermatite atopique canine et ou que l'élévation des taux d'IgE sériques totaux sont souvent non-associés à la dermatite atopique.

RESUMEN
Introducción – Las concentraciones totales de IgE son más altas en perros que en humanos. La infección persistente con larvas de *Toxocara canis* es prevalente en perros y se asocia con fuertes reacciones específicas de anticuerpos. Sin embargo, no se ha evaluado una correlación entre los niveles totales de anticuerpos IgE y anticuerpos específicos de *T. canis* en perros.
Objetivos – Determinar la relación entre la IgE total, la IgG e IgE específica de *T. canis* y los niveles de IgE específicos de alérgenos en perros atópicos y no atópicos, y para evaluar posibles factores de confusión.
Animales – suero de 30 perros atópicos y 30 perros no atópicos de propietarios privados.
Métodos – IgE total, anticuerpos específicos para *T. canis* y niveles de IgE específicos de alérgenos se evaluaron mediante ELISA.
Resultados – La IgE total, anticuerpos específicos para *T. canis* y los niveles de IgE específica de alérgeno fueron significativamente más altos en no atópicos en comparación con los perros atópicos. Se demostró una correlación positiva entre IgG específica de *T. canis* y IgE específica de *T. canis*, IgG específica de *T. canis* e IgE total, IgE específica de *T. canis* e IgE total, IgE específica de alérgeno e IgE total. No se detectaron diferencias en función de la edad, el sexo, la vacunación, la desparasitación o la época del año entre los perros atópicos y no atópicos. El tratamiento inmunomodulador previo y la causa de la atopia no influyeron en los niveles de anticuerpos de los perros atópicos.
Conclusiones – la IgE específica de *Toxocara canis* parece ser un componente principal de la IgE total en perros. Los niveles de IgE total y de *T. canis* son más altos en perros no atópicos que en los atópicos. Se especula que la infección por *T. canis* puede tener un efecto protector contra el desarrollo de dermatitis atópica canina y/o que las elevaciones en el nivel total de IgE sérica a menudo no están asociadas con la dermatitis atópica.

Zusammenfassung
Hintergrund – Die Gesamt IgE Konzentrationen sind bei Hunden höher als bei Menschen. Eine persistierende *Toxocara canis* Larveninfestation zeigt bei Hunden eine Prävalenz und wird mit einer starken Antikörperreaktion in Zusammenhang gebracht. Eine Korrelation zwischen Gesamt IgE und *T. canis*-spezifischen Antikörperlevels bei Hunden wurde bisher nicht evaluiert.
Ziele – Eine Bestimmung der Beziehung zwischen Gesamt IgE, *T. canis*-spezifischen IgG und IgE, sowie Allergen-spezifischen IgE Levels bei atopischen und nicht-atopischen Hunden und eine Evaluierung möglicher anderer verwirrender Faktoren.
Tiere – Sera von 30 atopischen und 30 nicht-atopischen Hunden in Privatbesitz.
Methoden – Gesamt IgE, *T. canis*-spezifische Antikörpern und Allergen-spezifische IgE Werte wurden mittels ELISA evaluiert.
Ergebnisse – Gesamt IgE, *T. canis*-spezifische Antikörper und Allergen-spezifische IgE Werte waren signifikant höher bei nicht-atopischen im Vergleich zu atopischen Hunden. Eine positive Korrelation wurde jeweils zwischen *T. canis*-spezifischem IgG und *T. canis*-spezifischem IgE, *T. canis*-spezifischem IgG und Gesamt IgE, *T. canis*-spezifischem IgE und Gesamt IgE, sowie zwischen Allergen-spezifischem IgE und Gesamt IgE festgestellt. Auf der Basis von Alter, Geschlecht, Impfung, Entwurmung oder Saison wurden zwischen atopischen und nicht-atopischen Hunden keine Unterschiede festgestellt. Eine frühere immunmodulatorische Behandlung und die Ursache der Atopie beeinflussten die Antikörperwerte der atopischen Hunde nicht.
Schlussfolgerungen – *T. canis*-spezifisches IgE scheint eine Hauptkomponente des Gesamt IgEs der Hunde zu sein. Gesamt und *T. canis*-spezifische IgE Werte liegen bei nicht-atopischen Hunden höher als bei atopischen Hunden. Es wird spekuliert, dass eine *T. canis*-Infektion eine schützende Wirkung auf die Entwicklung der atopischen Dermatitis der Hunde haben kann und/oder dass Erhöhungen der Gesamtserum IgE Werte oft nicht mit einer atopischen Dermatitis in Zusammenhang gebracht werden können.

要約 – 背景 -犬ではヒトよりも総IgE濃度が高いこと知られている。*Toxocara canis*の持続的な幼虫感染は犬で頻繁に認められ、強力かつ特異的な抗体反応と関連している。しかし、犬の総IgEと*T. canis*特異的抗体レベルとの相関は評価されていない。
目的 - 本研究の目的はアトピー犬および非アトピー犬における総IgE、*T. canis*特異的IgGおよびIgE、ならびにアレルゲン特異的IgEレベルの関連について検討するとともに、予測可能な交絡因子を検討することである。
動物 -飼育されているアトピー犬および非アトピー犬30頭から血清を採取して本研究に供した。
方法 - 総IgE、*T. canis*特異的抗体およびアレルゲン特異的IgEレベルをELISAによって評価した。
結果 - 総IgE、*T. canis*特異的抗体およびアレルゲン特異的IgE抗体価は、アトピー犬よりも非アトピー犬で有意に高値を示した。 *T. canis*特異的IgGと*T. canis*特異的IgE、*T. canis*特異的IgGと総IgE、*T. canis*特異的IgEと総IgE、ならびにアレルゲン特異的IgEと総IgE間の間では正の相関が認められた。アトピー犬および非アトピー犬の間では、年齢、性別、ワクチン接種、駆虫または季節に差異は認めなかった。過去に実施された免疫調節療法やアトピーの原因による、アトピー犬の抗体値への影響は認められなかった。
結論 - *Toxocara canis*特異的IgEは、犬における総IgEの主要な構成要素であると考えられる。総IgE抗体価ならびに*T. canis*特異的IgE抗体価は、アトピー犬よりも非アトピー犬で高値を示した。以上の結果から、*T. canis*感染に犬アトピー性皮膚炎の発症を予防する可能性か、総血清IgE抗体価の上昇がアトピー性皮膚炎に関連しないか、あるいはその双方が推測された。

摘要
背景 – 与人相比,犬体内的总IgE浓度更高。犬弓蛔虫幼虫对犬的持续感染十分普遍,且能引起强烈的特异性抗体反应。然而,犬总IgE与犬弓蛔虫特异性IgE抗体水平之间的关系尚未被评估过。
目的 - 为了明确在异位性皮炎和非异位性皮炎犬体内,总IgE水平、犬弓蛔虫特异性IgG 和 IgE水平,以及犬抗原特异性IgE水平几者之间的关系,并且评估可能混淆的因素。
动物 – 30异位性皮炎患犬和30只非异位性皮炎私家犬。
方法 - 通过ELISA评估犬总IgE、犬弓蛔虫特异性抗体和抗原特异性IgE三者的水平。
结果 – 相比于异位性皮炎患犬,非异位性皮炎犬体内总IgE、犬弓蛔虫特异性抗体和抗原特异性IgE三者的水平显著提高。结果表示,犬弓蛔虫特异性IgG 和犬弓蛔虫特异性 IgE水平,犬弓蛔虫特异性IgG和总IgE水平,犬弓蛔虫特异性 IgE和总IgE水平,犬抗原特异性IgE和总IgE水平,这几项之间均呈现正相关。在这两类犬中,年龄、性别、疫苗接种、驱虫或季节并没有发现差异。先前的免疫调节治疗和异位性皮炎的病因,并没有影响异位性皮炎犬的抗体水平。
结论 – 犬弓蛔虫特异性 IgE看起来是犬总 IgE的主要成分。相比异位性皮炎患犬,非异位性皮炎犬体内的总IgE、犬弓蛔虫特异性IgE的水平更高。据推测,犬弓蛔虫的感染也许对抑制犬异位性皮炎的发展有支持作用,且总血清IgE水平的升高常与异位性皮炎无关。

Resumo
Contexto – As concentrações de IgE total são mais altas em cães que em humanos. A infecção persistente por larvas de *Toxocara canis* é prevalente em cães e é associada a fortes reações específicas de anticorpos. Entretanto, a correlação entre IgE total e anticorpos *T. canis*-específicos ainda não foi avaliada.
Objetivos – Determinar a relação entre IgE total, anticorpos IgG e IgE *T. canis*-específicos, e os níveis de IgE alérgeno-específicos em cães atópicos e não-atópicos, e avaliar possíveis fatores de confusão.
Animais – Soro de 30 cães atópicos e 30 cães não-atópicos. Todos os cães eram de clientes.
Métodos – IgE total, anticorpos *T. canis*-específicos e IgE alérgeno-específicos foram avaliados por ELISA.
Resultados – IgE total, anticorpos *T. canis*-específicos e IgE alérgeno-específicos foram significativamente mais altos em cães não-atópicos comparado aos atópicos. Uma correlação positiva foi demonstrada entre IgG *T. canis*-específicos e IgE *T. canis*-específicos, IgG *T. canis*-específicos e IgE total, IgE *T. canis*-específicos e IgE total, e IgE alérgeno-específicos e IgE total. Não foram detectadas diferenças relacionadas a idade, gênero, status vacinal, vermifugação ou estação do ano entre cães atópicos e não-atópicos. Terapia imunomoduladora tópica e a causa da atopia também não influenciaram os níveis de anticorpos em cães atópicos.
Conclusões – Os anticorpos IgE *Toxocara canis*-específicos parecem ser o principal componente do IgE total de cães. Os níveis de IgE total e IgE *T. canis*-específicos são mais altos em cães não-atópicos comparado aos atópicos. Especula-se que a infecção por *T. canis* possa ter um efeito protetor contra o desenvolvimento da dermatite atópica canina e/ou que elevações nos níveis de IgE total frequentemente não estão associados à dermatite atópica.

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Anhang

Table S1: Factors influencing total IgE and *T. canis* L3 E/S-specific antibodies in atopic and healthy dogs

Atopic dog					**Healthy dog**				
No.	**Deworming**	**Vaccination updates**	**Season**	**ID and/ or ASIT**	**No.**	**Deworming**	**Vaccination updates**	**Season**	**In estrus**
1	Yes	Yes	May	ID	31	Yes	No	October	
2	Yes	Yes	April		32	No	No	October	
3	Yes	Yes	May	ID	33	Yes	Yes	May	Yes
4	Yes	Yes	May	ID	34	Yes	Yes	May	
5	No	No	September	ID	35	Yes	Yes	November	
6	Yes	Yes	November	ID	36	Yes	Yes	May	
7	Yes	Yes	November	ID/ ASIT	37	Yes	Yes	November	
8	Yes	Yes	November	ID	38	Yes	Yes	September	
9	Yes	Yes	November	ASIT	39	Yes	Yes	April	Yes
10	Yes	Yes	November	ID	40	Yes	Yes	May	
11	Yes	Yes	November	ID/ ASIT	41	No	No	July	
12	Yes	Yes	November	ID	42	No	No	September	
13	Yes	Yes	October		43	Yes	Yes	May	Yes
14	Yes	Yes	October	ID	44	Yes	No	April	
15	No	Yes	November		45	Yes	Yes	November	
16	No	Yes	November	ID/ ASIT	46	Yes	Yes	September	
17	Yes	Yes	November	ID	47	Yes	Yes	September	
18	No	No	December		48	No	Yes	November	Yes
19	No	Yes	December	ID	49	Yes	Yes	September	
20	No	Yes	December		50	Yes	Yes	October	
21	No	Yes	May		51	Yes	Yes	May	
22	Yes	Yes	May		52	Yes	No	April	
23	Yes	Yes	May		53	Yes	No	April	
24	Yes	Yes	May	ID	54	Yes	Yes	September	
25	No	No	May		55	Yes	Yes	September	
26	No	No	June		56	Yes	Yes	November	
27	Yes	Yes	August	ID	57	Yes	No	April	
28	Yes	Yes	September		58	Yes	Yes	May	Yes
29	Yes	No	August		59	Yes	Yes	June	
30	Yes	Yes	August	ASIT	60	Yes	Yes	October	

ID Immunomodulatory drugs; ASIT Allergen-specific immunotherapy